Enzymatic Assay of NADH OXIDASE

PRINCIPLE:

$$NADH + H^+ + O_2 \xrightarrow{\text{NADH Oxidase}} NAD + H_2O_2$$

CONDITIONS: T = 30°C, pH = 7.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 250 mM Potassium Phosphate Buffer, pH 7.0 at 30°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 30°C with 1 M KOH.)

B. 1 mM Flavin Adenine Dinucleotide Solution (FAD)
(Prepare 10 ml in deionized water using Flavin Adenine Dinucleotide, Disodium Salt, Sigma Prod. No. F-6625.)

C. 2.0 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (NADH)
(Dissolve the contents of one 10 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Sigma Stock No. 340-110, in the appropriate volume of deionized water. **PREPARE FRESH**.)

D. 30 mM Potassium Phosphate Buffer with 0.1% (w/v) Bovine Serum Albumin, pH 7.5 at 30°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 7.5 at 30°C with 1 M KOH.)

E. NADH Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 unit/ml of NADH Oxidase in cold Reagent D.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Deionized Water	1.90	1.90
Reagent A (Buffer)	0.60	0.60
Reagent B (FAD)	0.30	0.30
Reagent C (NADH)	0.10	0.10

Mix by inversion and equilibrate to 30°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Enzyme Solution)	0.10	------
Reagent D (Enzyme Diluent)	------	0.10

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3)(df)}{(6.22)(0.10)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.10 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of ß-NADH per minute at pH 7.0 at 30°C.

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FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final assay concentrations are
51 mM potassium phosphate, 0.07 mM ß-nicotinamide adenine dinucleotide, reduced form, 0.1 mM flavin adenine dinucleotide, 0.003% (w/v) bovine serum albumin, and 0.02 unit NADH oxidase.

REFERENCE:

Reusch, V.M., Burger, M.M. (1974) *Journal of Biological Chemistry* **249**, 5337-5345

NOTES:

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.